

A LIST OF BACTERIAL FLORA RESIDING IN THE MID AND HINDGUT REGIONS OF SIX SPECIES OF CARRION BEETLES (COLEOPTERA: SILPHIDAE)

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ABSTRACT: Forty-eight carrion beetles (Silphidae: *Nicrophorus tomentosus*, *Nicrophorus orbicollis*, *Oiceoptoma noveboracense*, *Oiceoptoma inaequale*, *Necrophila americana*, *Necrodes surinamensis*) were dissected and the midgut, hindgut, and associated hemolymph were cultured for bacteria. Eight specimens of each of the six species above were utilized. Analytical profile index rapid biochemical systems were used for bacterial identifications. Thirty-two bacteria were identified to species and seven to genus level. Six isolations were limited to "group" identifications and eight morphologically distinct bacteria could not be identified using the rapid biochemical test strips because of data base limitations. Many of the species are known opportunistic pathogens.

Most entomologists, and many field naturalists, have been aware of the existence of carrion beetles for many years and, in fact, the last quarter century has seen a fair number of publications on this family in entomological literature. Only recently, however, has a serious effort been made to survey the medically important true bacteria associated with the silphids. In that study (Solter *et al.* 1989) 36 carrion beetles (Silphidae: *Nicrophorus tomentosus* Weber, *Oiceoptoma noveboracense* (Forster), *Necrophila americana* (L.)) were collected in the Great Swamp National Wildlife Refuge (GSNWR), Basking Ridge, N.J. They were dissected and the midgut, hindgut and associated hemolymph were cultured for bacteria. Nineteen bacteria were identified to species and seven to the genus level. Several of the identified coliform and staphylococci bacteria were known opportunistic pathogens.

The primary objective of the current study was to expand on Solter *et al.* (1989), establishing the full spectrum of intestinal bacterial microflora harbored among a greater number of silphid species. In our study we were able to collect six of the eight species of silphids found in GSNWR (Shubeck 1983). In addition to the three species studied by Solter *et al.* (1989) our study included also *Nicrophorus orbicollis* Say, *Oiceoptoma inaequale* (F.), and *Necrodes surinamensis* (F.). Beetle taxonomy follows Anderson (1982). These six species are common at different times throughout the spring and summer months, in a variety of habitats including fields and forests. It was hoped such information

¹ Received June 30, 1993. Accepted July 20, 1993

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would help further define an epidemiological role for the carrion beetles.

A secondary objective of this study was to develop and utilize bacteriological techniques suitable for this type of study. Efforts to identify the large numbers and varieties of intestinal bacteria of silphid beetles have proven a formidable task. Colonies representing different species may not always be readily distinguishable when viewed among polymicrobial growth. This approach contrasts with that taken in a clinical microbiology laboratory, where efforts are directed only at identifying or characterizing suspected causative agents of infection. Isolation media commonly employed in clinical applications were used here to assist in the detection of such pathogens as *Salmonella*, *Shigella*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Staphylococcus aureus*, and pathogenic streptococci and clostridia. These media were also used in conjunction with biochemical tests as a means of grouping isolates.

Identification of pure culture isolates was accomplished using the API 20E, Rapid-STREPT, STAPH-Trac, STAPH-Ident and AN-IDENT identification systems. Such commercially available systems offer advantages over conventional methods including ease of use, standardized selection of biochemical tests, relatively low cost and quick response. Profile numbers are constructed from the results obtained and compared with those in an established data base. It should be understood that data bases supporting each of these systems have been derived primarily from human clinical strains. While systems like API are often used to identify isolates of non-human origin, there is a possibility of obtaining profile numbers either not contained in the data base, or of those yielding incorrect identifications. In spite of this, since bacteria associated with carrion beetles may be expected to represent those of entomological, veterinary or other environmental origins, we considered this study of sufficient importance to be pursued.

MATERIALS AND METHODS

Collection. Six species of carrion beetles (Silphidae) were collected at the Great Swamp National Wildlife Refuge in Basking Ridge, N.J. Collection was accomplished by the use of 5 ground surface type traps described elsewhere (Shubeck 1976). Three of these traps were placed along a straight line at 5 meter intervals within a mixed-oak forest, one in an adjacent field, and the fifth near a swamp. Each trap was baited with a fresh (1-7 days) and a stale (8-14 days) chicken leg, each in a separate styrofoam cup. Seven collections were made during the summer of 1988. Collecting dates were; 06/06, 06/13, 06/20, 06/27, 07/08, 08/02, 08/08.

Collected beetles were stored in a refrigerator and dissected within 72 hours. Only those that were alive at the time of dissection were used. The beetles were killed by crushing their heads with forceps, then rinsed in 2% Amphyl disinfectant and rinsed twice in sterile distilled water. They were then dissected and the midgut, hindgut and associated hemolymph were removed and placed in vials containing 1.0 ml of Trypticase Soy Broth with 15% glycerol. At least 8 specimens of each of the following 6 species were prepared: *Necrophila americana*, *Oiceoptoma inaequale*, *Oiceoptoma noveboracense*, *Nicrophorus orbicollis*, *Nicrodes surinamensis* and *Nicrophorus tomentosus*. The cultures were then stored at or below -60° C.

Culture Techniques. The following protocol is based on the work of Solter *et al.* (1989) but does include some changes which increased the likelihood of identifying additional bacterial species. The contents of each vial were thawed, homogenized and preincubated for 2 hours in lactose broth to facilitate cellular repair. Ten µL aliquots were then inoculated into Gram Negative (GN) Broth, Selenite Cysteine Medium and 0.1% sterile peptone water. Two drops of the preincubated homogenate were also plated onto Columbia Colistan Naladixic Acid (CNA) Blood Agar Plate (BAP), Mannitol Salt Agar and anaerobic media. The anaerobic media included Anaerobic Phenylethyl Alcohol Blood Agar Plates (ANA PEA BAP), Anaerobic Kanamycin-Vancomycin Blood Agar Plates (ANA KV BAP). These were cultured in a Gas Pak pouch at 30-35° C. The GN Broth was then plated onto the following agar plates; Salmonella Shigella (SS), Xylose Lysine Deoxycholate (XLD), MacConkey's (MAC), Levine Eosin Methylene Blue (EMB), Bismuth Sulfite (BS), Pseudosel and Cefsulfodin Irgasen Novobiocin Agar Base (*Yersinia* selective agar) (CIN agar). The inoculated tubes of 0.1% peptone water were further serially diluted and plated onto Trypticase Soy Agar (TSA) for total aerobic counts (TAC's). Aerobic plates were incubated at 35° C for 24 hours except for the TAC's which were incubated for 72 hours.

Representative colonial types from the primary isolation plates were then subcultured onto fresh media to obtain pure cultures. All gram negative organisms were streaked onto TSA, gram positive onto Columbia CNA BAP, and anaerobes onto ANA PEA BAP. Stock cultures of the purified isolates were prepared and stored at -60° C. Each gram negative isolate was streaked onto MAC, XLD, BS, and CIN agars. The plates were incubated and the morphological characteristics of the colonies determined. Tubes of Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), and Urease Broth were inoculated and then incubated.

Gram positive bacteria were characterized and grouped on the basis of colonial morphology observed on Columbia CNA BAP, TSA and coagulase and reactions in Oxidation Fermentation (OF) Glucose Bile Esculin and Trypticase Soy Broth (TSB) with 6.5% NaCl and the results were recorded. Anerobic isolates were tested for oxygen tolerance by incubating PEA BAP under both aerobic and anerobic conditions.

Final identification was performed using the following API identification systems; API 20E for gram negative bacteria, API Staph-Ident and Staph-Trac (catalase positive gram + cocci), API Rapid-Strep (catalase negative gram + cocci) and API An-Ident for anerobes.

RESULTS

Most of the beetles were collected during the four weeks of June, with the exception of *N. tomentosus* which was common during July and early August. Nineteen of the 48 beetles tested (39.6%) yielded aerobic counts on the order of 10^7 cfu's (colonies) per specimen, while 22 beetles (45.8%) yielded 10^6 cfu's per specimen, 6 beetles (12.5%) yielded 10^5 cfu's per specimen and 1 (2%) yielded 10^4 cfu's per specimen. There was no observable correlation between count and carrion beetle species. Overall, 607 isolates consisting of at least 42 different strains of bacteria were recovered. Of these isolates 52.1% were gram negative bacteria (21 strains), 21.1% were coagulase-negative staphylococci (5 strains), 8.1% were obligately anaerobic bacteria (7 strains), 7.6% were streptococci (6 strains), 5.4% were *Bacillus* spp., 4.4% were *Aerococcus* and less than 1% were coryneform bacteria (3 strains) or *Gemella* (formerly *Streptococcus*) *haemolysans*. A variety of colony types were recorded as either *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp. or *Bacillus* spp., so that the total number of varying strains recovered was actually more than indicated.

Facultative and Aerobic Gram Positive Bacteria.

Columbia CNA BAP's supported growth of all recovered gram-positive bacteria, while that of gram-negatives was effectively inhibited. Yeast colonies, although not targeted in this study, also grew on this media. Mannitol Salt Agar was considerably more selective, as only *Staphylococcus sciuri*, *S. xylosus* and *S. warneri* were recovered.

Biochemical testing performed on the gram-positive isolates was able to distinguish between the mannitol positive and mannitol negative non-agglutinating staphylococci, and between *Streptococcus/Aerococcus* spp. and *Streptococcus bovis* (Table 1). These tests were

used in conjunction with colonial morphology, to group the isolates prior to attempts at final identification.

Coagulase-negative staphylococci accounted for 52.9% of the 242 facultative and aerobic gram-positive bacteria recovered. Included were *Staphylococcus xylosus* (19.0%), *S. sciuri* (13.6%), *S. warneri* (9.1%), *S. saprophyticus* (8.3%), and 2.9% identified as *Staphylococcus* spp. (Table 1).

Streptococci accounted for 19.8% of all gram-positive bacteria, 15.7% of which were equally divided between the enterococci and those identified as *Streptococcus* spp. Also recovered were *Streptococcus sanguis* (2.1%), *S. lactis* (1.7%), and one *S. bovis* variant isolate. Two isolates were identified as *Gemella haemolysans*, a species previously assigned to the genus *Streptococcus*.

Another 13.6% of the gram-positive isolates consisted of a number of morphologically distinct catalase-positive, spore-forming rods, all of which were recorded as *Bacillus* spp.. Finally, a small number of coryneform bacteria (1.7%) were recovered.

Anaerobic Bacteria

Most of the 49 anaerobic isolates recovered belonged to the genus *Clostridium*, with 44.4% identified as *Clostridium* spp., 18.4% as *C. barati*, 14.3% as *C. bifermentans* and only one isolate each (2%) of *C. cadaveris* and *C. innocuum*. All clostridia failed to grow, or grew only slightly when incubated under aerobic conditions. The two remaining anaerobes recovered were *Peptostreptococcus anaerobius* (16.3%) and *Bacteroides capillosus* (2%) (Table 2).

Facultative and Aerobic Gram-Negative Bacteria

There were 23 different strains of gram-negative bacteria recovered, 14 of which belong to the family *Enterobacteriaceae*. The gram-negative bacteria constituted the single largest and most diverse group in this study. There was a total of 638 colonies recorded from among the 7 different gram-negative isolation plates, representing 514 isolates when only one species per plate is considered. The difference may represent either strain variation, or inadvertent recording (as different colony types) of a single strain more than once from the same plate. When counting each species only once per beetle specimen, the number of isolates drops to 316.

There were 23 different taxa and groups of gram-negative bacteria identified by the API 20E system (Table 3). Approximately one half of these constituted almost 90% of the 316 recovered isolates. The single

largest group was the so-called Proteeae tribe, including *Proteus* (26.6%), *Providencia* (16.5%) and *Morganella* (6.6%). Other predominant gram-negatives included *Serratia* (17.1%), *Citrobacter freundii* (9.5%), *Klebsiella* (7%) and *Hafnia alvei* (6.3%).

DISCUSSION

Bacteria representing most of the major groups, of which approximately 50% are gram-negative rods, were recovered from the beetles studied. These findings are consistent with previous studies of silphids (Solter *et al.* 1989) and other insects (Steinhaus 1941). Eighteen of the 26 bacterial types recovered from the Solter *et al.* study (1989) are included among the 45 different types recovered here. Frank pathogens were not recovered from either study with the exception of 3 possible *Salmonella* isolations in this study. On the other hand, many of these isolates are considered to be opportunistic pathogens, recognizing the often tenuous distinction between "harmless" and "pathogenic" microorganisms.

In the discussion that follows only species **not** mentioned in the Solter *et al.* study (1989) will be covered.

Gram Positive Bacterial Isolates (Table 1).

In animals, variants of *Staphylococcus* have evolved to be adapted to various hosts. These separate biotypes of ecotypes vary dependent upon the host species. Most of these organisms are coagulase negative and have limited clinical importance (Joklik *et al.* 1988). Included would be *Staphylococcus sciuri* which has been isolated from the skin of rodents, ungulates, carnivora and marsupials. It may be isolated from other mammals and environmental sources such as soil and water. It has only rarely been isolated from humans (Sneath *et al.* 1986).

Streptococcus faecalis is primarily located in the gastrointestinal tract of humans, homothermic and poikilothermic animals and in insects and plants. It is common in many non-sterile foods and its presence is often not related to fecal contamination. It is an opportunistic pathogen agent in urinary tract infections (Sneath *et al.* 1986). It also has been frequently associated with biliary tract infections, septicemia, wound infection and intraabdominal abscess complicating appendicitis, especially in the elderly or those who are immunologically compromised (Joklik *et al.* 1988).

Streptococcus sanguis is one of a group of streptococci that is associated with the oral cavity. These organisms are consistently isolated as a part of the flora of the mouth and are associated, along with several

other streptococcal species, in the production of dental plaque and dental carries. They have been isolated from the blood and heart valves in some cases of bacterial endocarditis. They are present in low levels in human feces and have been isolated from soil (Sneath *et al.* 1986).

Streptococcus bovis is frequently isolated from the alimentary tract of cows, sheep, and other ruminants and the feces of pigs. It is occasionally found in large numbers in human feces. It is one of the acidogenic streptococci found in raw and pasteurized milk, cream and cheese. It has been found occasionally in cases of endocarditis and is considered to be related to *S. faecalis* (Sneath *et al.* 1986).

Streptococcus lactis is non-pathogenic and is associated with the production of acid from large numbers of sugars. It is responsible for the souring of milk, and production of yogurt (Sneath *et al.* 1986).

As the name implies, *Streptococcus avium* is characteristically isolated from the feces of chickens and other fowl. These organisms also have been found in the feces of humans, dogs and pigs. They have been associated with appendicitis, otitis and abscesses of the brain (Sneath *et al.* 1986).

Aerococcus viridans is frequently found as a common airborne organism. Another variety is a marine organism which causes disease in lobsters. No pathogenic association is known to exist in humans (Sneath *et al.* 1986).

Gemella haemolysans is considered to be similar to the neisseria species. It is not known to be a pathogen. This organism has been isolated from bronchial secretions and human gingiva (Sneath *et al.* 1986).

Anaerobic Bacterial Isolates (Table 2)

Bacteroides capillosus is a gram (-) anaerobic organism forming tan to black pigments. *Bacteroides* are indigenous to various locations throughout the body including the mouth and thoracic region, intraabdominal and pelvic regions. The infections that they may cause are related to their location in the body (Krieg & Holt 1984).

Bacteroides capillosus has been isolated from cysts and wounds in humans, as well as the human mouth and feces. This organism has also been isolated from the intestinal tracts of several animals including hogs, mice and termites. It has also been found in sewage sludge (Krieg & Holt 1984).

Clostridia are anaerobic spore forming bacilli that are usually gram (+). Most species are obligate anaerobes. The pathogenic species produce soluble toxins some of which are extremely potent. The clostridia are widely distributed in nature and are present in soil and as inhabitants of the intestinal tract of humans and other animals (Joklik *et al.* 1988).

The histotoxic clostridia cause a severe infection of muscle—commonly called gas gangrene. Because the clostridia are so widely distributed in nature, contamination of wounds by these bacteria is very common. Often more than one clostridial species is present including both saprophytic and histotoxic species. The most commonly isolated histotoxic *Clostridium* is *C. perfringens*. However, several other species are commonly encountered in soft tissue infections, abscesses, wound infections, anaerobic cellulitis and gas gangrene. These organisms may be considered non-pathogenic, but they can be opportunistic pathogens. Included in this group are *C. barati*, *C. bifermentans*, *C. innocuum*, and *C. cadaveris*. While these organisms may not produce toxins, they may play a synergistic role in the development of gas gangrene (Joklik *et al.* 1988).

Peptostreptococcus anaerobius is an anaerobic gram (+) coccus. These organisms are a part of the normal flora of the mouth, gastrointestinal tract and genital tract. They are particularly important in pleuropulmonary disease, brain abscesses and obstetric and gynecologic infections (Sneath *et al.* 1986).

Gram Negative Bacterial Isolates (Table 3)

The bulk of the species in this category are members of family Enterobacteriaceae. They are glucose fermenting bacilli or coccobacilli and as the name implies they are enteric bacteria;

Serratia marcescens is a prominent opportunistic pathogen affecting hospitalized patients. At one time they were thought to be harmless saprophytes and were used to trace air currents in the environment and in hospitals. In nature they are found widely distributed in soil and water and are found associated with a large number of plants and animals, including insects. Almost all *Serratia* infections are associated with underlying disease. They cause nosocomial infections of the urinary tract and wound infections, pneumonia and septicemia. Mastitis in cows and other animal infections are caused by this organism (Joklik *et al.* 1988).

Serratia liquifacens has been isolated from clinical specimens. The disease spectrum for this species is similar to *S. marcescens* (Joklik *et al.* 1988).

Klebsiella pneumoniae is normally found in the intestinal tract of man and animals, but in lower numbers than *E. coli*. As the name suggests it can cause pneumonia. It can also infect other sites than the respiratory tract. It is the mucoid capsule that determines the pathogenicity. The organism is an opportunistic pathogen and usually causes illness in a patient who is already compromised. It can also cause urinary

tract and wound infections, bacteremia and meningitis (Joklik *et al.* 1988).

Klebsiella oxytoca resembles *K. pneumoniae* in disease spectrum and it is also very similar from a clinical viewpoint (Joklik *et al.* 1988).

Klebsiella ozonae causes chronic atrophic rhinitis characterized by a fetid odor. Nasal and pharyngeal infections are primarily seen in people from endemic regions in Eastern Europe and South America. It can also be isolated from urinary tract and soft tissue infections and from secondary bacteremia (Joklik *et al.* 1988).

Enterobacter cloacae is found less frequently than *Klebsiella* and *E. coli*. It is most frequently associated with urinary tract infections in nosocomial patients having other underlying problems. In the 1970's *Enterobacter agglomerans* and *E. cloacae* were responsible for 150 bacteremias and 9 deaths in a nationwide epidemic caused by contaminated intravenous fluids (Joklik *et al.* 1988).

Hafnia alvei is found in feces of man and other animals including birds. It is also found in sewage, soil and water. The infections it produces are similar to those produced by *Enterobacter* (Krieg & Holt 1984).

Some serotypes of *Salmonella* are primarily adapted to one species of host or another. *Salmonella pullorum* is adapted to poultry rather than man and is primarily transmitted between poultry. Humans can, however, develop salmonellosis from contaminated food and water (Krieg & Holt 1984).

Cedacea spp. is an enteric genus that has been isolated from a variety of opportunistic infections (over 50% from the respiratory tract). The organisms are infrequent opportunistic pathogens and constitute only a small percentage of isolates. Very little is known about their ecology, epidemiology or role in human disease (Krieg & Holt 1984).

Mollerella wisconsinensis is a recently described organism and was formerly considered in Enteric Group 46. It has been isolated from feces. The reported isolates have been found in patients with diarrhea but there is no evidence that it can actually cause diarrhea (Farmer *et al.* 1985).

Tatumella ptyseos is similar to other members of the Enterobacteriaceae. It has been isolated from human clinical specimens—86% from the respiratory tract. It may be a rare opportunistic pathogen, whose epidemiology is not known (Krieg & Holt 1984).

The remaining gram negative bacterial isolates are all non-fermenting bacilli:

Pseudomonas fluorescens is found in soil and water. It is commonly associated with spoilage of foods, such as eggs, meats and it is often iso-

lated from clinical specimens (Krieg & Holt 1984). Although these organisms are not etiological agents of disease, they may be the cause of opportunistic infections of wounds and the urinary tract (Krieg & Holt 1984).

Pseudomonas testosteroni occurs in soil. It is not considered pathogenic, but like *P. fluorescens* it may be opportunistic (Krieg & Holt 1984).

Pasteurella are parasitic on the mucous membrane of the upper respiratory and digestive tracts of mammals (rarely man) and birds. Some primary diseases include hemorrhagic septicemia of cattle and fowl cholera in chicken, turkeys, ducks, etc. These organisms also cause secondary pneumonia-like illness in cattle and sheep (Krieg & Holt 1984).

The most significant source of microorganisms colonizing the silphid gut is the carrion on which they feed. The bacteria recovered in this study should, therefore, be reflective of those commonly found on decaying carcasses. Most of the recovered species are, in fact, widely distributed in nature and capable of existing as free-living organisms. The variety of gram-negative and gram-positive, facultative and anaerobic bacteria recovered from all six species indicates conditions which may be favorable to at least transient populations of pathogenic bacteria. Such organisms may be acquired exogenously from infected carrion, in which case the silphids could become vectors of disease transmission.

Table 1. The number of times each identified gram positive bacterial species was isolated from six silphid species. N.a. = *Necrophila americana*, N.o. = *Nicrophorus orbicollis*, O.n. = *Oiceoptoma noveboracense*, O.i. = *Oiceoptoma inaequale*, N.t. = *Nicrophorus tomentosus*, N.s. = *Necrodes surinamensis*. (Silphidae—8 specimens per species were used.)

GRAM POSITIVE BACTERIAL ISOLATE	N.a.	N.o.	O.n.	O.i.	N.t.	N.s.	TOTALS
<i>Staphylococcus xylosus</i> Schleifer and Kloos	6	8	8	8	8	8	46
<i>Staphylococcus sciuri</i> Kloos, Schleifer and Smith	8	6	4	3	7	5	33
<i>Staphylococcus warneri</i> Kloos and Schleifer	5	6	3	0	2	6	22
<i>Staphylococcus saprophyticus</i> (Fairbrother)	3	2	3	0	8	4	20
<i>Staphylococcus</i> spp.	2	1	1	0	3	0	7
<i>Streptococcus faecalis</i> Andrewes and Horder	3	3	3	0	3	6	18
<i>Streptococcus sanguis</i> White and Niven	3	0	1	1	0	0	5
<i>Streptococcus lactis</i> (Lister) Lohnis	0	0	1	3	0	0	4
<i>Streptococcus avium</i> Nowlan and Deibel	0	0	1	0	0	0	1
<i>Streptococcus bovis</i> variant Orla-Jensen	0	1	0	0	0	0	1
<i>Streptococcus</i> spp.	0	2	6	5	3	3	19
<i>Aerococcus viridans</i> Williams, Hirsch and Cowan	1	2	4	5	7	8	27
<i>Bacillus</i> spp.	7	8	8	6	4	0	33
coryneform bacteria	0	0	2	0	1	1	4
<i>Gemella haemolysans</i> (Thjotta and Boe) Berger	1	0	0	0	1	0	2
TOTALS	39	39	45	31	47	41	242

Table 2. The number of times each identified anaerobic bacterial species was isolated from six silphid species. N.a. = *Necrophila americana*, N.o. = *Nicrophorus orbicollis*, O.n. = *Oiceoptoma noveboracense*, O.i. = *Oiceoptoma inaequale*, N.t. = *Nicrophorus tomentosus*, N.s. = *Necrodes surinamensis*. (Silphidae—8 specimens per species were used.)

ANAEROBIC BACTERIAL ISOLATE	N.a.	N.o.	O.n.	O.i.	N.t.	N.s.	TOTALS
<i>Clostridium barati</i> Prevot	5	4	0	0	0	0	9
<i>Clostridium bifermentans</i> Weinberg and Seguin	4	1	2	0	0	0	7
<i>Clostridium cadaveris</i> Klein	0	0	1	0	0	0	1
<i>Clostridium innocuum</i> Smith and King	0	0	1	0	0	0	1
<i>Clostridium</i> spp.	2	2	5	4	5	4	22
<i>Peptostreptococcus anaerobius</i> Natvig	0	0	3	5	0	0	8
<i>Bacteroides capillosus</i> (Tissier) Kelly	0	1	0	0	0	0	1
TOTALS	11	8	12	9	5	4	49

Table 3. The number of times each identified gram negative bacterial species was isolated from six silphid species. N.a. = *Necrophila americana*, N.o. = *Nicrophorus orbicollis*, O.n. = *Oiceoptoma noveboracense*, O.i. = *Oiceoptoma inaequale*, N.t. = *Nicrophorus tomentosus*, N.s. = *Necrodes surinamensis*. (Silphidae—8 specimens per species were used.)

GRAM NEGATIVE BACTERIAL ISOLATE	N.a.	N.o.	O.n.	O.i.	N.t.	N.s.	TOTALS
<i>Proteus mirabilis</i> Hauser	8	8	6	5	8	8	43
<i>Proteus vulgaris</i> Hauser	8	7	7	5	6	8	41
<i>Providencia rettgeri</i> (Hadley, Elkins, and Caldwell)	3	3	6	6	8	8	34
<i>Providencia alcalifacens</i> (De Dalles Gomes)	3	0	3	0	4	8	18
<i>Morganella morganii</i> (Winslow, Kligler, and Rothberg)	1	1	6	8	0	5	21
<i>Serratia marcescens</i> Bizio	5	5	1	1	0	0	12
<i>Serratia liquifacens</i> Grimes and Hennerty	3	0	1	1	4	0	9
<i>Serratia</i> spp.	6	2	4	5	8	8	33
<i>Citrobacter freundii</i> (Braak)	5	7	6	6	4	2	30
<i>Klebsiella pneumoniae</i> (Schroeter) Trevisan	3	5	2	0	7	3	20
<i>Klebsiella oxvtoca</i> (Flugge)	1	1	0	0	0	0	2
<i>Hafnia alvei</i> Moller	3	0	6	2	6	3	20
<i>Alcaligenes</i> spp.	0	0	3	4	0	0	7
<i>Alcaligenes</i> or <i>Morganella</i> spp.	0	0	3	0	0	0	3
<i>Pasteurella</i> or <i>Acinetobacter</i> spp.	0	0	1	2	0	0	3
<i>Enterobacter cloacae</i> (Jordan) Hormaeche and Edwards	0	0	1	2	0	0	3
<i>Pasteurella</i> spp.	0	0	0	1	0	0	1
<i>Salmonella pullorum</i> Rettger or <i>Hafnia alvei</i> Moller	0	0	0	0	2	1	3
<i>Cedacea</i> spp. or <i>Klebsiella ozonae</i> (Abel) Bergey	0	0	1	0	0	0	1
<i>Pseudomonas fluorescens</i> Migula	0	0	1	0	0	0	1
<i>Pseudomonas testosteroni</i> Marcus and Talalay or <i>Pasteurella</i> spp.	0	0	0	1	0	0	1
<i>Tautumella pytyeos</i> Hollis, Hickman and Fanning	0	0	1	0	0	0	1
<i>Mollerella wisconsinensis</i> (Farmer <i>et al.</i>)	0	0	1	0	0	0	1
No Match	2	2	0	3	1	0	8
TOTALS	51	41	60	52	58	54	316

ACKNOWLEDGMENTS

We thank William Koch and Janith Taylor for permission to collect at the Great Swamp National Wildlife Refuge, Basking Ridge, N.J.

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